

96. (Amended) The method of claim 76, wherein the [functional] additional domain is heterologous with respect to the two nucleic acid-binding domains.

### **Remarks**

Claims 40-70 and 72-88 are pending. Claim 40 has been amended to correct for typographical errors. Claims 57, 58, 63, 65, 76, 77, 94 and 96 have been amended for improved clarity. No new matter has been added.

Cancellation and/or amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The cancellation and/or amendments to the claims are being made solely to expedite prosecution of the present application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

### **Rejection of claims 40-70, 72, 89-92, 94-95, and 97 under 37 C.F.R. § 103 (a) in view of Park et al., Mitchell et al., Harrison and Schultz**

Claims 40-70, 72, 89-92, 94-95, and 97 have been rejected under 37 C.F.R. § 103 (a) as being unpatentable over Park et al. (*PNAS* 89: 9094 (1992)), in view of Mitchell et al. (*Science* 245:371 (1989)), Harrison (*Nature* 353:715 (1991)), and Schultz (*Nature* 240: 426 (1988)). Applicants respectfully traverse this rejection.

Claim 40, and claims 41-65 and 72 dependent therefrom, are drawn to a nucleic acid encoding a chimeric protein which binds to a nucleic acid comprising a composite binding site, the chimeric protein comprising two nucleic acid-binding domains, each of which binds a sequence which is a portion of the composite binding site, and wherein only one of the two nucleic acid-binding domains includes a zinc finger motif. Claim 66, and claims 67-70 dependent therefrom, are drawn to a nucleic acid encoding a chimeric protein which binds to a nucleic acid comprising a composite binding site, the chimeric protein comprising two nucleic acid-binding domains, each of which binds a sequence which is a portion of the composite binding site, and wherein only one of the two nucleic acid-binding domains is a nucleic acid-binding domain from a homeodomain containing protein.

Park et al. is relied on by the Examiner as teaching "a general strategy for designing proteins to recognize specific DNA-binding sites" and that "[t]his technique creates a protein that recognizes the composite site (page 9094, column 1)." The Examiner indicates that Park et al. "do not teach to specifically use the DNA-binding domains from distinct families of nucleic acid binding domains, use of specific types of domains such as zinc-finger domains."

Mitchell et al. is relied on by the Examiner as teaching that "different DNA binding transcription factors are composed of a surprising variety of usually separable DNA binding and transcriptional activation domains (page 372, column 2)."

Harrison is relied on as teaching that "many DNA-binding proteins recognize specific sites through small, discrete domains and that these domains can be interchanged between proteins, showing that these domains are independent folded units."

Schultz is relied on as teaching that "enzymes can be created by adding or replacing entire binding or catalytic domains to generate hybrid enzymes with novel specificities" and that "[s]elective fusion of nucleic acid-specific binding domains may produce sequence-specific DNA or RNA cleaving enzymes (page 431, column 1)."

It is the Examiner's position that

[i]t would have been obvious to one of skill in the art at the time the invention was made to use the various DNA binding domains, activation domains, and cleavage domains taught by Mitchell et al., Harrison, and Schulz in the general strategy for designing proteins to recognize specific DNA-binding sites taught by Park et al. because Park et al. teach that it is within the ordinary skill in the art to stitch the DNA binding domains together from any proteins that recognize a specific DNA sequence by binding along the major groove, to recognize a composite site and Mitchell et al., Harrison, and Schultz teach such domains that can be functionally separated and recombined with other domains.

Applicants submit that the cited references alone, or together with the general knowledge in the art at the time the invention was filed, fail to provide neither sufficient motivation to combine them to obtain the claimed nucleic acids nor the requisite reasonable expectation of success.

However, applicants did not simply infer, as asserted in the Office Action, that "because one of the references in the rejection did not teach the whole invention, there would not have been a reasonable expectation of success." On the contrary, Applicants' noted that Park et al. fail to provide any motivation to combine the references and to provide a reasonable expectation of success, and that none of the secondary references cited cured this defect.

The heart of the Examiner's position appears to be that Park et al. alone "provides very strong motivation to make chimeric DNA-binding proteins that bind to composite sequences by fusing two previously separate DNA binding domains together." Applicants disagree.

Applicants respectfully submit that the Examiner's conclusion requires one to read the excerpted Park et al sentences out of context. In view of the actual disclosure of the Park et al reference, a person of skill in the art would understand the general statements made in Park et al. to

refer to arms of dimeric DNA binding proteins, and would not give broader meaning to statements in that reference that could otherwise be given broader interpretation, i.e., if taken out of that context.

There are several lines of evidence that even Park et al. did not intend the general statements in their paper to be given the broader interpretation reflected in the office action.

First, Park et al.'s later published paper (*PNAS* 90: 4892 (1993); copy of which is enclosed herein as Exhibit A), defines the concept of protein stitchery, referring to their '92 paper, as representing that "individual basic arms (half sites) of the dimer and the individual half sites of the DNA can be recombined or stitched together in various sequences to form new proteins selective for binding to the new DNA sites" (emphasis added; see paragraph bridging pages 4892 and 4893). Thus, Park et al. defined the general concept of protein stitchery as combining "arms" of proteins which normally form dimers.

Second, if Park et al. truly intended the generic meaning represented by the Examiner, they wouldn't have limited themselves to proteins which bind along the major groove of the DNA. Clearly there are DNA binding proteins which bind along the minor groove and indeed homeodomains and zinc finger domains, such as are used to illustrate the subject invention, do make minor groove contacts.

In addition, the publication of applicants' work in *Science*, as discussed further below, belies acceptance in the art of broader conclusions from the Park et al. reference.

Accordingly, Applicants again submit that the general statements made by Park et al. would not have been viewed, and should not now be viewed, as being as broad as the Examiner contends, and would not provide the requisite motivation to make the claimed chimeric proteins.

Moreover, as Applicants explained before, Parks et al. cross-linked together the DNA binding domains of two proteins which normally bind DNA only in the form of a homodimer. However, Park et al. does not teach or suggest that a chimeric protein having a composite DNA binding domain consisting of two or more DNA binding domains from different types of DNA binding proteins, which do not normally interact with each other, would bind DNA with higher affinity to the composite binding site than to portions of it. Harrison and Mitchell et al. merely teach that DNA binding domains can be separated from the rest of DNA binding proteins. However, these teachings do not fail to cure the defect of Park et al. Furthermore, neither Harrison nor Mitchell refer to portions of DNA binding domains, e.g., individual zinc finger domains, which can also constitute part of Applicants' claimed composite DNA binding domain.

The Examiner also states that "[a]bsent evidence to the contrary, there would have been a reasonable expectation of success that the domains taught by Mitchell et al. and Harrison could be combined with each other to create a protein that recognizes a composite binding site as taught by

Park et al." However, as set forth above, Mitchell and Harrison merely teach that DNA binding domains can be separated from the rest of DNA binding proteins. There was no reasonable expectation of success that a chimeric protein containing a composite DNA binding domain consisting of DNA binding domains from proteins which are unrelated, as claimed by Applicants, would bind to a composite DNA binding site with higher affinity than to each of the half sites to which each of the DNA binding domains of the composite DNA binding domain bind. Nor was there any reasonable expectation of success that binding of such a chimeric protein containing a transcriptional activation domain to a DNA binding site would be able to stimulate transcription of a target gene operably linked to the DNA binding site, as Applicants showed.

Further support that there was no reasonable expectation of success to obtain the claimed invention is provided by a statements made by one of the inventors of the instant application after publication of their invention in the journal *Science*: "laboratory tests have proved the artificial switch can find, and control, a single gene among the 80,000 that exists in humans." The article (attached hereto as Exhibit B) also quotes Carl Pabo, as stating "the critical thing was showing it can bind the proper site." Furthermore, if there had been a reasonable expectation of success, Applicants' description of the invention would not have been published in the prestigious peer-reviewed *Science* journal (Pomerantz et al. (1995) *Science* 267: 93, attached hereto as Exhibit C).

With regard to making a nucleic acid and vector comprising the nucleic acid which encodes the chimeric protein, the Examiner states that "it would have been obvious to do so because Parks et al. teach that a continuous approximately 70 amino-acid protein that should recognize a predictable site can be made, instead of using a cysteine linker, and thus it would have been obvious to make a nucleic acid that encodes this protein and place the nucleic acid in a vector to express the protein, because such a way of making a mutated, recombinant protein is and was well known in the art." Applicants respectfully traverse this statement. Although Parks et al. may make general statements, i.e., speculate, that the cross-linking between the two monomer DNA binding domains (presumably of a dimeric DNA binding protein) could be replaced by a peptide bond, a person of skill in the art at the time the invention was made would have known that a peptidic bond may give rise to a protein structure that is different from that resulting from disulfide cross-linking. Thus, there was no reasonable expectation of success that two different DNA binding domains linked together through a peptidic bond would form a chimeric DNA binding protein.

Thus, in view of all of the above, Applicants respectfully request that the Examiner reconsider and withdraw rejection of claims 40-72 under 37 C.F.R. § 103 (a) as being unpatentable over Park et al., in view of Mitchell et al., Harrison, and Schultz.

**Rejection of claims 40-70 and 72-88 under 37 C.F.R. § 103 (a) in view of Park et al., Mitchell et al., Harrison, Schultz and Gossen et al.**

Claims 40-70 and 72-88 have been rejected under 37 C.F.R. § 103 (a) as being unpatentable over Park et al. (supra), in view of Mitchell et al. (supra), Harrison (supra), Schultz (supra) as applied to claims 40-70, 72, 89-92, 94-95, and 97 above, and further in view of Gossen et al. (U.S. Patent No. 5, 464,758). Applicants respectfully traverse this rejection.

Claim 40 and claims 41-65, and 72-74 dependent therefrom are drawn to a nucleic acid encoding a chimeric protein which binds to a nucleic acid comprising a composite binding site, the chimeric protein comprises two nucleic acid-binding domains, each of which binds a sequence which is a portion of the composite binding site, and wherein only one of the two nucleic acid-binding domains includes a zinc finger motif. Claim 66 and claims 67-70 dependent therefrom are drawn to a nucleic acid encoding a chimeric protein which binds to a nucleic acid comprising a composite binding site, the chimeric protein comprises two nucleic acid-binding domains, each of which binds a sequence which is a portion of the composite binding site, and wherein only one of the two nucleic acid-binding domains is a nucleic acid-binding domain from a homeodomain containing protein. Claims 75-83 and 84-89 are drawn to a method for modulating expression of a gene in a cell, comprising modulating the level of a chimeric protein in a cell which includes a gene operably to a composite binding site to which the chimeric protein binds, wherein the chimeric protein comprises two nucleic acid-binding domains, each of which binds a sequence which is a portion of the composite binding site

Park et al., Mitchell et al., Harrison and Schultz are relied upon by the Examiner as disclosing what is summarized in the previous section. Gossen et al. is relied upon by the Examiner as teaching "a nucleotide molecule coding for a chimeric transactivator fusion protein comprising a DNA binding domain (tet repressor binding domain) and a transactivation domain (such as VP16 of HSV)."

It is the Examiner's opinion that "[i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to form a transcriptional regulatory system from the DNA encoding a chimeric transactivation protein made obvious by the teachings of Park et al. (AW2), Mitchell et al. (S), Harrison (T) and Schultz (U), using the method taught by Gossen et al. because Gossen et al. teach that it is within the ordinary skill in the art to make a nucleic acid vector that encodes a chimeric transactivator fusion protein (under the control of a promoter active in eukaryotic cells), make a nucleic acid encoding a heterologous protein operably linked to a regulator binding site that the chimeric protein binds to..."

Applicants respectfully submit that, as set forth above, Park et al. (AW2), Mitchell et al. (S), Harrison (T) and Schultz (U) do not make obvious a chimeric transactivation protein. Thus, even

if, as contended by the Examiner, Gossen et al. teach that "it is within the skill in the art to make a nucleic acid vector that encodes a chimeric transactivator fusion protein (...), make a nucleic acid encoding a heterologous protein operably linked to a regulator binding site that the chimeric proteins binds to, place the nucleic acid in a eukaryotic cell..." the cited references fail to provide any motivation to combine the references and to provide a reasonable expectation of success.

Thus, in view of all of the above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 40-70 and 72-88 as being unpatentable over Park et al., in view of Mitchell et al., Harrison, Schultz and further in view of Gossen et al.

### **Conclusion**

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,  
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